



PATENT APPLICATION

1.132

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :  
OKADA et al. :  
Serial No.: 10/031,404 : Group Art Unit: 2114  
Filed: May 15, 2002 : Examiner: JIANG, SHAOJIA A  
For: PHARMACEUTICALS FOR NEUROPATHIC PAIN

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## DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, Masamichi OKADA, a citizen of Japan, hereby declare and state:

I graduated from Tokyo Institute of Technology with receiving Ph.  
D. in neurochemistry in March of 1985.

In April of 1985, I was employed by Yamanouchi Pharmaceutical  
Co., Ltd.

Since then up to the present, I have been engaged in the  
neurochemistry research and development.

I am a member of the Society for Neuroscience, U.S.A.

I published some literatures and patent applications including:

- 1) T. Kato, M. Okada, T. Nakano, T. Nagatsu, J. Emura, S.  
Sakakibara, Y. Iizumi, S. Tsushima, N. Kakazawa and H. Ogawa, The  
presence of substance P carboxy-terminal hepatapeptide in pig brain stem.  
Prog. Japan Acad. 56 388-393 (1980).

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- 2) T. Kato, M. Okada and T. Nagatsu, Distribution of post-proline cleaving enzyme in human brain and the peripheral tissues. **Mol. Cellular Biochem.** 32 117-121 (1980).
- 3) T. Hama, M. Okada, K. Kojima, T. Kato, M. Matsuyama and T. Nagatsu, Purification of dipeptidyl-aminopeptidase IV from human kidney by anti dipeptidyl-aminopeptidase IV affinity chromatography. **Mol. Cellular Biochem.** 43 35-42 (1982).
- 4) K. Koshiya, M. Okada, K. Imai, T. Kato, T. Tanaka, H. Hatanaka and T. Kato, Localization of angiotensin-converting enzyme, prolyl endopeptidase and other peptidases in cultured neuronal or glial cells. **Neurochem. Int.** 7 125-130 (1985).
- 5) M. Okada and T. Kato, Peptidase-containing neurons in rat striatum. **Neurosci. Res.** 2 421-433 (1985).
- 6) M. Okada\*, Effects of a new thyrotropin releasing hormone analogue, YM14673, on the in vivo release of acetylcholine as measured by intracerebral dialysis in rats. **J. Neurochem.** 56, 1544-1547 (1991).
- 7) M. Shimizu-Sasamata, M. Yamamoto, M. Okada, T. Yamaguchi and T. Tamura, Effects of indeloxazine hydrochloride on behavioral and biochemical changes in the chronic phase of focal cerebral ischemia in rats. **Arch. int. Pharmacodyn.** 314, 74-88 (1991).
- 8) M. Yamamoto, M. Ooyama, Y. Ozawa, M. Okada, S. Tada, T. Yamaguchi and H. Endoh, Effects of indeloxazine hydrochloride, a cerebral activator, on passive avoidance learning impaired by disruption of cholinergic transmission in rats. **Neuropharmacology** 32, 695-701 (1993).

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- 9) M. Yamamoto, K. Takahashi, M. Ohyama, M. Sasamata, S. Yatsugi, M. Okada and H. Endoh, Possible involvement of central cholinergic system in ameliorating effects of indeloxazine, a cerebral activator, on disturbance of learning behavior in rats. **Prog. Neuro-Psychopharmacol. & Biol. Psychiat.** 18, 603-613 (1994).
- 10) J. Ohmori, S. Sakamoto, H. Kubota, M. Shimizu-Sasamata, M. Okada, S. Kawasaki, K. Hidaka, J. Togami, T. Furuya and K. Murase, 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione hydrochloride (YM90K) and related compounds: structure-activity relationships for the AMPA-type non-NMDA receptor. **J. Med. Chem.** 37, 467-475 (1994).
- 11) J. Ohmori, H. Kubota, M. Shimizu-Sasamata, M. Okada and S. Sakamoto, Novel  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionate receptor antagonists: synthesis and structure-activity relationships of 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-pyrido[2,3-*b*]pyrazine-dione and related compounds. **J. Med. Chem.** 39, 1331-1338 (1996).
- 12) M. Shimizu-Sasamata, S. Kawasaki-Yatsugi, M. Okada, S. Sakamoto, S. Yatsugi, J. Togami, K. Hatanaka, J. Ohmori, K. Koshiya, S. Usuda and K. Murase, YM90K: pharmacological characterization as a selective and potent  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor antagonist. **J. Pharmacol. Exp. Ther.** 276, 84-92 (1996).
- 13) M. Okada\*, A. Kohara and T. Yamaguchi, Characterization of YM90K, a selective and potent antagonist of AMPA receptors, in rat cortical mRNA-injected *Xenopus* oocytes. **Eur. J. Pharmacol.** 309, 299-306 (1996).

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- 14) S. Kawabata, R. Tsutsumi, A. Kohara, T. Yamaguchi, S. Nakanishi and M. Okada\*, Control of calcium oscillations by phosphorylation of metabotropic glutamate receptors. *Nature* 383, 89-92 (1996).
- 15) M. Matsumoto, T. Nomura, K. Momose, Y. Ikeda, Y. Kondou, H. Akiho, Y. Kimura, M. Okada and T. Yamaguchi, Inactivation of a novel neuropeptide Y/peptide YY receptor gene in primate species. *J. Biol. Chem.* 271, 27217-27220 (1996).
- 16) J. Ohmori, M. Shimizu-Sasamata, M. Okada and S. Sakamoto, Novel AMPA receptor antagonist: synthesis and structure-activity relationships of 1-hydroxy-7-(1*H*-imidazol-1-yl)-6-nitro-2,3(1*H*,4*H*)-quinoxalinedione and related compounds. *J. Med. Chem.* 39, 3971-3979 (1996).
- 17) M. Matsumoto, K. Hidaka, H. Akiho, S. Tada, M. Okada and T. Yamaguchi, Low stringency hybridization of the dopamine D4 receptor revealed D4-like mRNA distribution of the orphan seven-transmembrane, API, in human brain. *Neurosci. Lett.* 219, 1-4 (1996).
- 18) J. Ohmori, M. Shimizu-Sasamata, M. Okada and S. Sakamoto, 8-(1*H*-imidazol-1-yl)-7-nitro-4(5*H*)-imidazo-[1,2-*a*]quinoxalinone and related compounds: synthesis and structure-activity relationships for the AMPA-type non-NMDA receptor. *J. Med. Chemist.* 40, 2053-2063 (1997).
- 19) K. Nakahara, M. Okada and S. Nakanishi, The metabotropic glutamate receptor mGluR5 induces calcium oscillations in cultured astrocytes via protein kinase C phosphorylation. *J. Neurochem.* 69,

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1467-1475 (1997).

20) K. Ohno, M. Okada, R. Tsutsumi, A. Kohara and T. Yamaguchi, Kainate excitotoxicity is mediated by AMPA- but not kainate-preferring receptors in embryonic rat hippocampal cultures. **Neurochem. Int.** 31, 715-722 (1997).

21) S. Nakanishi, Y. Nakajima, M. Masu, Y. Ueda, K. Nakahara, D. Watanabe, S. Yamaguchi, S. Kawabata and M. Okada, Glutamate receptors: brain function and signal transduction. **Brain Res. Review** 26, 230-235 (1998)

22) K. Ohno, M. Okada\*, R. Tsutsumi, S. Sakamoto and T. Yamaguchi, The AMPA-receptor antagonist YM90K reduces AMPA receptor-mediated excitotoxicity in rat hippocampal cultures. **Jpn. J. Pharmacol.** 76 (1), 105-108 (1998)

23) A. Kohara, M. Okada\*, R. Tsutsumi, K. Ohno, M. Takahashi, M. Simizu-Sasama, J. Shishikura, H. Inami, S. Sakamoto and T. Yamaguchi, In Vitro characterization of YM872: a selective, potent and highly water-soluble  $\alpha$ -amino-3-hydroxy-5-methylimidazole-4-propionate (AMPA) receptor antagonist. **J. Pharmacy and Pharmacology** 50, 1-8 (1998)

24) K. Ohno, M. Okada, R. Tsutsumi, A. Kohara and T. Yamaguchi, Characterization of cyclothiazide-enhanced kainate excitotoxicity in rat hippocampal cultures. **Neurochem. Int.** 32, 265-271 (1998).

25) S. Kawabata, A. Kohara, R. Tsutsumi, H. Itahana, S. Hayashibe, T. Yamaguchi and M. Okada\*, Diversity of calcium signaling

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by metabotropic glutamate receptors. **J. Biol. Chem.** 273, 17381-17385 (1998).

26) K. Noda-Saita, M. Matsumoto, K. Hidaka, K. Hatanaka, J. Ohmori, M. Okada and T. Yamaguchi, Dopamine D4-like binding sites labeled by [3H]Nemonapride include substantial serotonin 5-HT<sub>2A</sub> receptors in primate cerebral cortex. **B. B. R. C.** 255, 367-370 (1999)

I am one of the inventors of the inventions described in the specification of the above-identified application (hereinafter, referred to as the "present application").

The following experiments were carried out by me, by other co-inventors of the present application, or under their immediate supervision.

**EXPERIMENTS****1) Test Compounds:**

Compound C: Compound of Example 92 of JP-A-2002-105085

Compound D: Compound of Example 40 of WO 02/062803

Compound E: Compound of Example 43 of WO 02/062803

Compound F: Compound of Example 17 of WO 02/062803

Compound G: Compound of Example 79 of Japanese patent application No. 2001-196750

The chemical structures of these test compounds are shown in the following table.

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**Page 7/10****2) Assay of mGluR1 inhibitory activity**

The binding affinity for mGluR1 was assayed in accordance with the test method in Test Example 1 in the present application. The binding affinity reflects the potency of mGluR1 antagonistic activity.

**3) Diabetic neuropathy model**

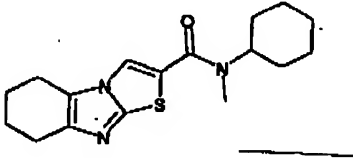
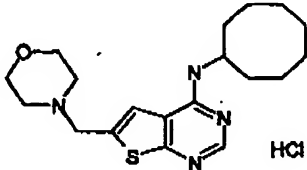
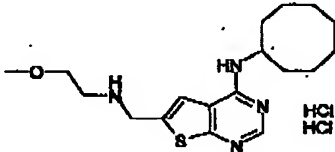
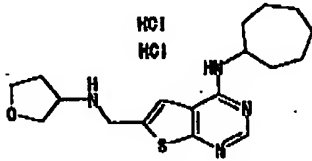
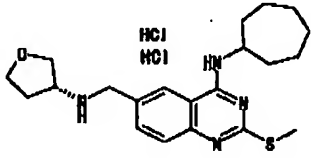
Effects of the test compounds *on the* *against tail-pinch stimulus* *M.O.* *12/26/2002*  
for ~~prolonged~~ latency in the diabetic neuropathy model were assessed in accordance with the test method in Test Example 2 in the present application.

**4) Nerve-ligated model**

Pain thresholds (allodynia-improving effects) against mechanical stimulus in nerve-ligated model were assessed in accordance with the test method in Test Example 3 in the present application.

Results of 1) and 2) above are shown in the following Table.

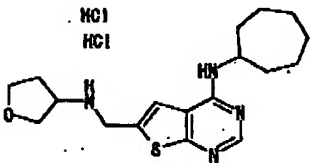
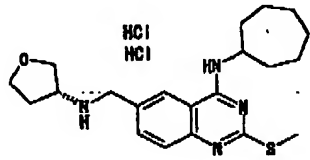
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Compound	IC <sub>50</sub>	Structure	Prolonged latency in diabetic neuropathy model
C	49 nM		effective at 30 mg/kg (intraperitoneal)
D	24 nM		improved at 30 mg/kg p.o.
E	74 nM		effective at 100 mg/kg p.o.
F	5 nM		effective at 10 mg/kg p.o.
G	2.7 nM		effective at 10 mg/kg p.o.



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Results of 3) above are shown in the following Table.

Compound	IC <sub>50</sub>	Structure	Allodynia-improving effect in Chung Model
F	5 nM		effective at 30 mg/kg p.o.
G	2.7 nM		effective at 30 mg/kg p.o.

5) From the above results, the present invention is not limited to the chemical structures of the compounds and can clearly show that neuropathic pain will be treated by systemic administration of a compound having an mGluR1 antagonistic activity.

6) Alleged Undue Experimentation

The above data shows a good correlation between the *in vitro* binding affinity and the *in vivo* effective dose. That is, it is easy to understand for one skilled in the art that at a dose <sup>of</sup> from 10<sup>μ</sup>g to 100 mg <sup>MO</sup> effective compounds in animal models can be found from selected mGluR1 antagonists having an IC<sub>50</sub> value of from about 3 nM to about 80 nM. That is, one skilled in the art is not required to conduct undue experiments for

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the selection of potential compounds for the treatment of neuropathic pain.

The specification of the present application describes that the compound having an activity of 0.1  $\mu$ M or less as an  $IC_{50}$  value is preferable and the dose is within the range of 1 to 1000 mg, wherein the data above are all included.

Accordingly, because one skilled in the art can select compounds having an mGluR1 antagonistic activity that is sufficient for the treatment of neuropathic pain by selecting the mGluR1 antagonists having an  $IC_{50}$  value of from about 3 nM to about 80 nM, one skilled in the art can select the compounds of the present invention without undue experiments and also can set the effective dose thereof.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: December 26, 2002

Masamichi Okada  
Masamichi OKADA